IFWO

RAW SEQUENCE LISTING   DATE: 09/16/2004
PATENT APPLICATION: US/10/822,370   TIME: 15:26:09

        Input Set : A:\BSA04-11.txt
        Output Set: N:\CRF4\09162004\J822370.raw

     3 <110> APPLICANT: Brookhaven Science Associates
     4   Shanklin, John
     5   Whittle, Edward J.
     7 <120> TITLE OF INVENTION: Mutant Fatty Acid Desaturase and Methods for Directed
Mutagenesis
     9 <130> FILE REFERENCE: CIP of 10/017,145 filed December 14, 2001 which was a CIP of
W--> 10 09/328,550 filed on June 9, 1999, which was a CIP of 09/233,856
W--> 11 filed on January 19, 1999
    13 <140> CURRENT APPLICATION NUMBER: 10/822,370
    14 <141> CURRENT FILING DATE: 2004-04-12
    16 <150> PRIOR APPLICATION NUMBER: 09/328,550
    17 <151> PRIOR FILING DATE: 1999-06-09
    19 <150> PRIOR APPLICATION NUMBER: 10/017,145
    20 <151> PRIOR FILING DATE: 2001-12-14
    22 <150> PRIOR APPLICATION NUMBER: 09/233,856
    23 <151> PRIOR FILING DATE: 1999-01-19
    25 <160> NUMBER OF SEQ ID NOS: 19
    27 <170> SOFTWARE: PatentIn version 3.2
    29 <210> SEQ ID NO: 1
    30 <211> LENGTH: 363
    31 <212> TYPE: PRT
    32 <213> ORGANISM: Ricinus communis
    35 <220> FEATURE:
    36 <221> NAME/KEY: misc_feature
    37 <223> OTHER INFORMATION: ricinus communis delta 9 18:0 Acyl ACP Desaturase
    39 <400> SEQUENCE: 1
    41 Ala Ser Thr Leu Lys Ser Gly Ser Lys Glu Val Glu Asn Leu Lys Lys
    42 1   5   10   15
    45 Pro Phe Met Pro Pro Arg Glu Val His Val Gln Val Thr His Ser Met
    46   20   25   30
    49 Pro Pro Gln Lys Ile Glu Ile Phe Lys Ser Leu Asp Asn Trp Ala Glu
    50   35   40   45
    53 Glu Asn Ile Leu Val His Leu Lys Pro Val Glu Lys Cys Trp Gln Pro
    54   50   55   60
    57 Gln Asp Phe Leu Pro Asp Pro Ala Ser Asp Gly Phe Asp Glu Gln Val
    58 65   70   75   80
    61 Arg Glu Leu Arg Glu Arg Ala Lys Glu Ile Pro Asp Asp Tyr Phe Val
    62   85   90   95
    65 Val Leu Val Gly Asp Met Ile Thr Glu Glu Ala Leu Pro Thr Tyr Gln
    66   100   105   110
    69 Thr Met Leu Asn Thr Leu Asp Gly Val Arg Asp Glu Thr Gly Ala Ser
    70   115   120   125
    73 Pro Thr Ser Trp Ala Ile Trp Thr Arg Ala Trp Thr Ala Glu Glu Asn
    74   130   135   140

```
 77 Arg His Gly Asp Leu Leu Asn Lys Tyr Leu Tyr Leu Ser Gly Arg Val
 78 145                 150                 155                 160
 81 Asp Met Arg Gln Ile Glu Lys Thr Ile Gln Tyr Leu Ile Gly Ser Gly
 82                 165                 170                 175
 85 Met Asp Pro Arg Thr Glu Asn Ser Pro Tyr Leu Gly Phe Ile Tyr Thr
 86                 180                 185                 190
 89 Ser Phe Gln Glu Arg Ala Thr Phe Ile Ser His Gly Asn Thr Ala Arg
 90             195                 200                 205
 93 Gln Ala Lys Glu His Gly Asp Ile Lys Leu Ala Gln Ile Cys Gly Thr
 94         210                 215                 220
 97 Ile Ala Ala Asp Glu Lys Arg His Glu Thr Ala Tyr Thr Lys Ile Val
 98 225                 230                 235                 240
101 Glu Lys Leu Phe Glu Ile Asp Pro Asp Gly Thr Val Leu Ala Phe Ala
102                 245                 250                 255
105 Asp Met Met Arg Lys Lys Ile Ser Met Pro Ala His Leu Met Tyr Asp
106                 260                 265                 270
109 Gly Arg Asp Asp Asn Leu Phe Asp His Phe Ser Ala Val Ala Gln Arg
110         275                 280                 285
113 Leu Gly Val Tyr Thr Ala Lys Asp Tyr Ala Asp Ile Leu Glu Phe Leu
114         290                 295                 300
117 Val Gly Arg Trp Lys Val Asp Lys Leu Thr Gly Leu Ser Ala Glu Gly
118 305                 310                 315                 320
121 Gln Lys Ala Gln Asp Tyr Val Cys Arg Leu Pro Pro Arg Ile Arg Arg
122                 325                 330                 335
125 Leu Glu Glu Arg Ala Gln Gly Arg Ala Lys Glu Ala Pro Thr Met Pro
126                 340                 345                 350
129 Phe Ser Trp Ile Phe Asp Arg Gln Val Lys Leu
130         355                 360
133 <210> SEQ ID NO: 2
134 <211> LENGTH: 1092
135 <212> TYPE: DNA
136 <213> ORGANISM: Ricinus communis
139 <220> FEATURE:
140 <221> NAME/KEY: misc_feature
141 <223> OTHER INFORMATION: residues 138 to 1239 of open reading frame
143 <400> SEQUENCE: 2
144 gcctctaccc tcaagtctgg ttctaaggaa gttgagaatc tcaagaagcc tttcatgcct      60
146 cctcgggagg tacatgttca ggttacccat tctatgccac cccaaaagat tgagatcttt     120
148 aaatccctag acaattgggc tgaggagaac attctggttc atctgaagcc agttgagaaa     180
150 tgttggcaac cgcaggattt tttgccagat cccgcctctg atggatttga tgagcaagtc     240
152 agggaactca gggagagagc aaaggagatt cctgatgatt attttgttgt tttggttgga     300
154 gacatgataa cggaagaagc ccttcccact tatcaaacaa tgctgaatac cttggatgga     360
156 gttcgggatg aaacaggtgc aagtcctact tcttgggcaa tttggacaag ggcatggact     420
158 gcggaagaga atagacatgg tgacctcctc aataagtatc tctacctatc tggacgagtg     480
160 gacatgaggc aaattgagaa gacaattcaa tatttgattg gttcaggaat ggatccacgg     540
162 acagaaaaca gtccatacct tgggttcatc tatacatcat ccaggaaag ggcaaccttc     600
164 atttctcatg gaacactgcc cgacaagcc aaagagcatg agacataaa gttggctcaa     660
166 atatgtggta caattgctgc agatgagaag cgccatgaga cagcctacac aaagatagtg     720
168 gaaaaactct ttgagattga tcctgatgga actgttttgg cttttgctga tatgatgaga     780
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```
170 aagaaaattt ctatgcctgc acacttgatg tatgatggcc gagatgataa tcttttttgac   840
172 cactttttcag ctgttgcgca gcgtcttgga gtctacacag caaaggatta tgcagatata   900
174 ttggagttct tggtgggcag atggaaggtg dataaactaa cggccctttc agctgaggga   960
176 caaaaggctc aggactatgt ttgtcggtta cctccaagaa ttagaaggct ggaagagaga   1020
178 gctcaaggaa gggcaaagga agcacccacc atgcctttca gctggatttt cgataggcaa   1080
180 gtgaagctgt ag                                                        1092
183 <210> SEQ ID NO: 3
184 <211> LENGTH: 34
185 <212> TYPE: DNA
186 <213> ORGANISM: Artificial
188 <220> FEATURE:
189 <223> OTHER INFORMATION: amplification primer
192 <220> FEATURE:
193 <221> NAME/KEY: misc_feature
194 <223> OTHER INFORMATION: PCR primer; sequence flanking unique XbaI site at the 5' end
of
195       the open reading frame
197 <400> SEQUENCE: 3
198 gtgagcggat aacaatttca cacagtctag aaat                                 34
201 <210> SEQ ID NO: 4
202 <211> LENGTH: 72
203 <212> TYPE: DNA
204 <213> ORGANISM: Artificial
206 <220> FEATURE:
207 <223> OTHER INFORMATION: amplification primer
210 <220> FEATURE:
211 <221> NAME/KEY: misc_feature
212 <222> LOCATION: (56)..(57)
213 <223> OTHER INFORMATION: PCR primer is a degenerate oligonucleotide in which "n"
indicates
214       the presence of either C, A, T or G at that nucleotide position
216 <400> SEQUENCE: 4
217 ccaaattgcc caagacgtcg gacttgcacc tgtttcatcc cgaactccat ccaamnnatt      60
219 cagcattgtt tg                                                         72
222 <210> SEQ ID NO: 5
223 <211> LENGTH: 31
224 <212> TYPE: DNA
225 <213> ORGANISM: Artificial
227 <220> FEATURE:
228 <223> OTHER INFORMATION: amplification primer
231 <220> FEATURE:
232 <221> NAME/KEY: misc_feature
233 <223> OTHER INFORMATION: PCR primer
235 <400> SEQUENCE: 5
236 gaaacaggtg caagtccgac gtcttgggca a                                    31
239 <210> SEQ ID NO: 6
240 <211> LENGTH: 26
241 <212> TYPE: DNA
242 <213> ORGANISM: Artificial
244 <220> FEATURE:
245 <223> OTHER INFORMATION: amplification primer
```

W--> (indicator at line 217)

```
248 <220> FEATURE:
249 <221> NAME/KEY: misc_feature
250 <223> OTHER INFORMATION: PCR primer
252 <400> SEQUENCE: 6
253 gttttctgtc cgcggatcca ttcctg                                          26
256 <210> SEQ ID NO: 7
257 <211> LENGTH: 34
258 <212> TYPE: DNA
259 <213> ORGANISM: Artificial
261 <220> FEATURE:
262 <223> OTHER INFORMATION: amplification primer
265 <220> FEATURE:
266 <221> NAME/KEY: misc_feature
267 <223> OTHER INFORMATION: PCR primer
269 <400> SEQUENCE: 7
270 gtgagcggat aacaatttca cacagtctag aaat                                 34
273 <210> SEQ ID NO: 8
274 <211> LENGTH: 30
275 <212> TYPE: DNA
276 <213> ORGANISM: Artificial
278 <220> FEATURE:
279 <223> OTHER INFORMATION: amplification primer
282 <220> FEATURE:
283 <221> NAME/KEY: misc_feature
284 <223> OTHER INFORMATION: PCR primer
286 <400> SEQUENCE: 8
287 cacgaggccc tttcgtcttc aagaattctc                                      30
290 <210> SEQ ID NO: 9
291 <211> LENGTH: 28
292 <212> TYPE: DNA
293 <213> ORGANISM: Artificial
295 <220> FEATURE:
296 <223> OTHER INFORMATION: amplification primer
299 <220> FEATURE:
300 <221> NAME/KEY: misc_feature
301 <223> OTHER INFORMATION: PCR primer
303 <400> SEQUENCE: 9
304 ttgataagtg ggaagggctt cttccgtt                                        28
307 <210> SEQ ID NO: 10
308 <211> LENGTH: 66
309 <212> TYPE: DNA
310 <213> ORGANISM: Artificial
312 <220> FEATURE:
313 <223> OTHER INFORMATION: amplification primer
316 <220> FEATURE:
317 <221> NAME/KEY: misc_feature
318 <222> LOCATION: (32)..(34)   ok
319 <223> OTHER INFORMATION: PCR primer is degenerate olignucleotide in which "n"
indicates
320        the presence of either C, A T, or G at that nucleotide position
```

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```
321           and in which "k" indicates either T or G
323 <220> FEATURE:
324 <221> NAME/KEY: misc_feature
325 <222> LOCATION: (41)..(43)
326 <223> OTHER INFORMATION: PCR primer is a degenerate oligonucleotide in which "n"
indicates
327           the presence of either C, A, T or G and in which "k" indicates
328           the presence of either T or G.
330 <220> FEATURE:
331 <221> NAME/KEY: misc_feature
332 <222> LOCATION: (44)..(46)
333 <223> OTHER INFORMATION: PCR primer is a degenerate oligonucleotide in which "n"
indicates
334           the presence of either C, A, T, or G at that nucleotide position
335           and in which "k" indicates the presence of either T or G.
337 <400> SEQUENCE: 10
W--> 338 aacggaagaa gcccttccca cttatcaaac annkctgaat npknnkgatg gagttcggga      60
340 tgaaac                                                                    66
343 <210> SEQ ID NO: 11
344 <211> LENGTH: 26
345 <212> TYPE: DNA
346 <213> ORGANISM: Artificial
348 <220> FEATURE:
349 <223> OTHER INFORMATION: amplification primer
352 <220> FEATURE:
353 <221> NAME/KEY: misc_feature
354 <223> OTHER INFORMATION: PCR primer
356 <400> SEQUENCE: 11
357 tccattcctg aaccaatcaa atattg                                             26
360 <210> SEQ ID NO: 12
361 <211> LENGTH: 70
362 <212> TYPE: DNA
363 <213> ORGANISM: Artificial
365 <220> FEATURE:
366 <223> OTHER INFORMATION: amplification primer
369 <220> FEATURE:
370 <221> NAME/KEY: misc_feature
371 <222> LOCATION: (22)..(24)
372 <223> OTHER INFORMATION: PCR primer in a degenerate oligonucleotide in which "n"
indicates
373           the presence of either C, A, T or G at that nucleotide position
374           and in which "k" indicates the presence of either T or G at that
375           nucleotide position.
377 <220> FEATURE:
378 <221> NAME/KEY: misc_feature
379 <222> LOCATION: (28)..(30)
380 <223> OTHER INFORMATION: PCR primer in a degenerate oligonucleotide in which "n"
indicates
381           the presence of either C, A, T or G at that nucleotide position
382           and in which "k" indicates the presence of either T or G at that
383           nucleotide position.
385 <220> FEATURE:
386 <221> NAME/KEY: misc_feature
```

RAW SEQUENCE LISTING ERROR SUMMARY   DATE: 09/16/2004
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Please Note:
Use of n and/or Xaa have been detected in the Sequence Listing. Please review the
Sequence Listing to ensure that a corresponding explanation is presented in the <220>
to <223> fields of each sequence which presents at least one n or Xaa.

Seq#:4; N Pos. 56,57
Seq#:10; N Pos. 32,33,41,42,44,45
Seq#:12; N Pos. 22,23,28,29,49,50

Invalid <213> Response:
Use of "Artificial" only as "<213> Organism" response is incomplete,
per 1.823(b) of New Sequence Rules. Valid response is Artificial Sequence.

Seq#:3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19

VERIFICATION SUMMARY   DATE: 09/16/2004
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Input Set : **A:\BSA04-11.txt**
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```
L:10 M:259 W: Allowed number of lines exceeded, <130> FILE REFERENCE:
L:11 M:259 W: Allowed number of lines exceeded, <130> FILE REFERENCE:
L:217 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:4 after pos.:0
L:338 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:10 after pos.:0
L:394 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:12 after pos.:0
```